

Rediscovery and phylogenetic analysis of *Agnostrup*, a least known genus of Mecistocephalidae (Chilopoda, Geophilomorpha) in China

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Abstract

The genus *Agnostrup* Foddai, Bonato, Pereira & Minelli, 2003 is one of the least known genera within the family Mecistocephalidae Bollman, 1893. In this study, we rediscovered the genus *Agnostrup* in China and provided a comprehensive redescription of *A. striatus* (Takakuwa, 1949). Additionally, we conducted a molecular phylogenetic analysis using CO1, 16S, and 28S sequence data, revealing a low genetic distance between *Agnostrup* and *Nannarrup* Foddai, Bonato, Pereira & Minelli, 2003. After comparing the morphological characteristics of these two genera, we synonymized the genus *Nannarrup* **syn. nov.** with *Agnostrup*. As a result, three species previously belonging to the genus *Nannarrup* were transferred to *Agnostrup*: *A. hoffmani* (Foddai et al., 2003), **comb. nov.**, *A. innuptus* (Tsukamoto, 2022), **comb. nov.**, and *A. oyamensis* (Tsukamoto, 2022), **comb. nov.**

Key Words

Agnostrup, description, Mecistocephalidae, molecular phylogeny, taxonomy

Introduction

The geophilomorph family Mecistocephalidae Bollman, 1893, recognized as a monophyletic basal clade, comprises approximately 170 species across 11 genera (Bonato et al. 2011). Within this family, three subfamilies are recognized: Mecistocephalinae Bollman, 1893; Dicellyphilinae Cook, 1896; and Arrupinae Chamberlin, 1912. The Arrupinae includes four valid genera: *Arrup* Chamberlin, 1912; *Agnostrup* Foddai, Bonato, Pereira & Minelli, 2003; *Partygarrupius* Verhoeff, 1939; and *Nannarrup* Foddai, Bonato, Pereira & Minelli, 2003 (Foddai et al. 2003).

Agnostrup Foddai, Bonato, Pereira & Minelli, 2003, characterized by forcipular trochanteropraefemur with a well-developed tooth and 41 leg-bearing segments, is one of the least-known genera in the family Mecistocephalidae Bollman, 1893 (Foddai et al. 2003). This genus includes only three species recorded from temperate regions

of East Asia: *A. striatus* (Takakuwa, 1949) from Shanxi, China; *A. paucipes* (Miyosi, 1955) from Matsuyama, Japan; and *A. striganovae* (Titova, 1975) from the nearby Sikhote-Alin mountains of Russia. Few articles about the morphology, distribution, ecology, or molecular phylogenetics of species from this genus have been documented since its original description. In particular, the type materials of *Taiwanella paucipes* and *Taiwanella striata* are considered to have been lost after examining many collections (Foddai et al. 2003; Uliana et al. 2007). The published accounts of its morphology are incomplete and contain ambiguous details, and the species' distinguishing features have not been thoroughly examined.

Recently, we collected four specimens from Shanxi, China, that obviously belong to the genus *Agnostrup*. Based on a critical evaluation of published information and examination of fresh material, these specimens were identified as *A. striatus*; one of these specimens is selected

and described here as the neotype for *Taiwanella striata*. We also revised the diagnostic characters of the genus *Agnostrop* and established the phylogenetic relationship of the family Mecistocephalidae based on the COI, 16S, and 28S sequence data. Molecular phylogenetics shows a close relationship between the genus *Agnostrop* and *Nannarrup* Foddai, Bonato, Pereira & Minelli, 2003. After comparing the morphological characters of these two genera, we synonymize the genus *Nannarrup* syn. nov. with *Agnostrop*.

Materials and methods

Biological sampling

Two adult male and two adult female specimens of *Agnostrop* spp. were collected from Shanxi Province, China (see additional geographic details below) in November 2023 and June 2024. These specimens were individually preserved in 75% ethanol and deposited in the herbarium of the National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences (CMMI).

Morphological terminology for external anatomy follows Bonato et al. (2010). Taxonomic characters were examined in lactic acid and photographed using a Leica M205 FCA stereomicroscope (7.8–160×) or an Olympus BX51 microscope (100–400×). The photos were converted into hand-drawn illustrations with SKETCHBOOK 6.0.6.

DNA extractions and fragment amplification

Two body segments from each sample were used for DNA extraction. Following the manufacturer's protocol, DNA was extracted using the DNeasy® Blood & Tissue Kit (QIAGEN GmbH, Germany). The polymerase chain reaction (PCR) was used to amplify the cytochrome c oxidase subunit I (COI), mitochondrial ribosomal gene 16S, and nuclear ribosomal DNA 28S fragments. PCR primers and programs are provided in Table 1.

Genetic distance and molecular phylogenetic analyses

The genetic distance among genera of Mecistocephalidae was calculated using the Kimura 2-parameter model in MEGA X (Kumar et al. 2018).

Maximum-likelihood (ML) tree were constructed using the concatenated dataset with IQ-tree 1.6.12 (Nguyen et al. 2015). GTR+I+G4+F was selected as the preferred substitution model for ML, with 1,000,000 ultrafast bootstrap replicates (Hoang et al. 2018). Bayesian inference (BI) was conducted using MRBAYES 3.2.6 (Ronquist et al. 2012), with 10,000,000 bootstrap replicates. The GTR+F+I+G4 was chosen as the preferred model for BI, sampling every 1,000 generations and using 25% of the

trees as burn-in. A split frequency of less than 0.01 was used to determine stationarity, and the consensus tree was constructed from the remaining trees.

Results

Molecular phylogenetic analyses

Sequences from the *Agnostrop striatus*, along with 16 other Mecistocephalidae samples from different genera and a sample from Geophilidae, were aligned. ML and BI analyses were utilized to construct phylogenetic trees for the combined COI+16S+28S dataset (Figs 1, 2). In the family Mecistocephalidae, the genera *Agnostrop*, *Anarrup*, *Arrup*, *Nannarrup*, and *Partygarrupius*, as well as the species *Dicelophilus anomalus* (Chamberlin, 1904), *Dicelophilus pulcher* (Kishida, 1928), and *Dicelophilus praetermissus* Tsukamoto & Eguchi, 2024, from the genus *Dicelophilus*, all have 41 leg-bearing segments. According to the phylogenetic tree, *Agnostrop* is the sister to *Nannarrup* (PP = 1; BS = 99%). While the genus *Arrup* is nested at the base of Mecistocephalidae in both ML and BI analyses (PP = 1). The genus *Dicelophilus* Cook, 1896 forms a clade with *Proterotaiwanella* + *Tygarrup* + *Mecistocephalus* in ML analyses, uncertain in BI analyses. The genus *Proterotaiwanella* Bonato, Foddai & Minelli, 2002 (subfamily Dicelophilinae) forms a clade with *Tygarrup* and *Mecistocephalus* (subfamily Mecistocephalinae) (PP = 1; BS = 85%), which is inconsistent with morphological results.

Genetic distance among *Agnostrop* and other mecistocephalidae genus

In examining genetic distances within the Mecistocephalidae family, we focused on the *Agnostrop* genus and its relationship with other genera, using sequences from COI, 16S, and 28S mitochondrial DNA regions (Table 3). The average K2P genetic distance is 20.0% (COI), 25.9% (16S), and 11.68% (28S). The data showed distinct genetic differentiation, with *Agnostrop* displaying high genetic distances from the *Tygarrup* genus, marked by 22.2% for COI, 26.9% for 16S, and 11.8% for 28S, indicating significant evolutionary divergence. In contrast, genetic distances between *Agnostrop* and *Nannarrup* genera were notably lower at 16.4% for COI, 18.1% for 16S, and 10.6% for 28S. The mean K2P genetic distance between the specimens of *Agnostrop striatus* is 12.7% (COI), 13.3% (16S), and 2% (28S), which is close to the intergeneric genetic distance between *Agnostrop* and *Nannarrup*. These lower distances suggest a close genetic relationship.

Morphological comparison of *Agnostrop* and *Nannarrup*

The genera *Agnostrop* and *Nannarrup* both belong to the subfamily Arrupinae and share the following diagnostic

Table 1. Primers and programs of PCR.

Loci	Primers Sequence 5– 3	Program	References
COI	LC01490 GGTCAACAAATCATAAAGATATTGG HCO2198 TAAACTTCAGGGTGACCAAAAATCA	2min at 94 °C; 35 cycles of 15s at 95 °C, 40s at 45–47 °C and 45s at 72 °C; 10min at 72 °C	Folmer et al. 1994; Joshi and Karanth 2011
16S	16Sar CGCCTGTTTATCAAAACAT 16Sb CTCCGGTTTGAAGTCAGATC	5min at 95 °C; 35 cycles of 30s at 95 °C, 30s at 55 °C and 1min at 72 °C; 3min at 72 °C	Xiong and Kocher 1991
28S	28SD1F GGGACTACCCCTGAATTTAAGCAT 28SrD4b CCTTGGTCCGTGTTTCAAGAC	3min at 95 °C; 35 cycles of 30s at 95 °C, 30s at 65 °C and 1min at 72 °C; 3min at 72 °C	Boyer and Giribet (2007); Edgecombe and Giribet (2006)

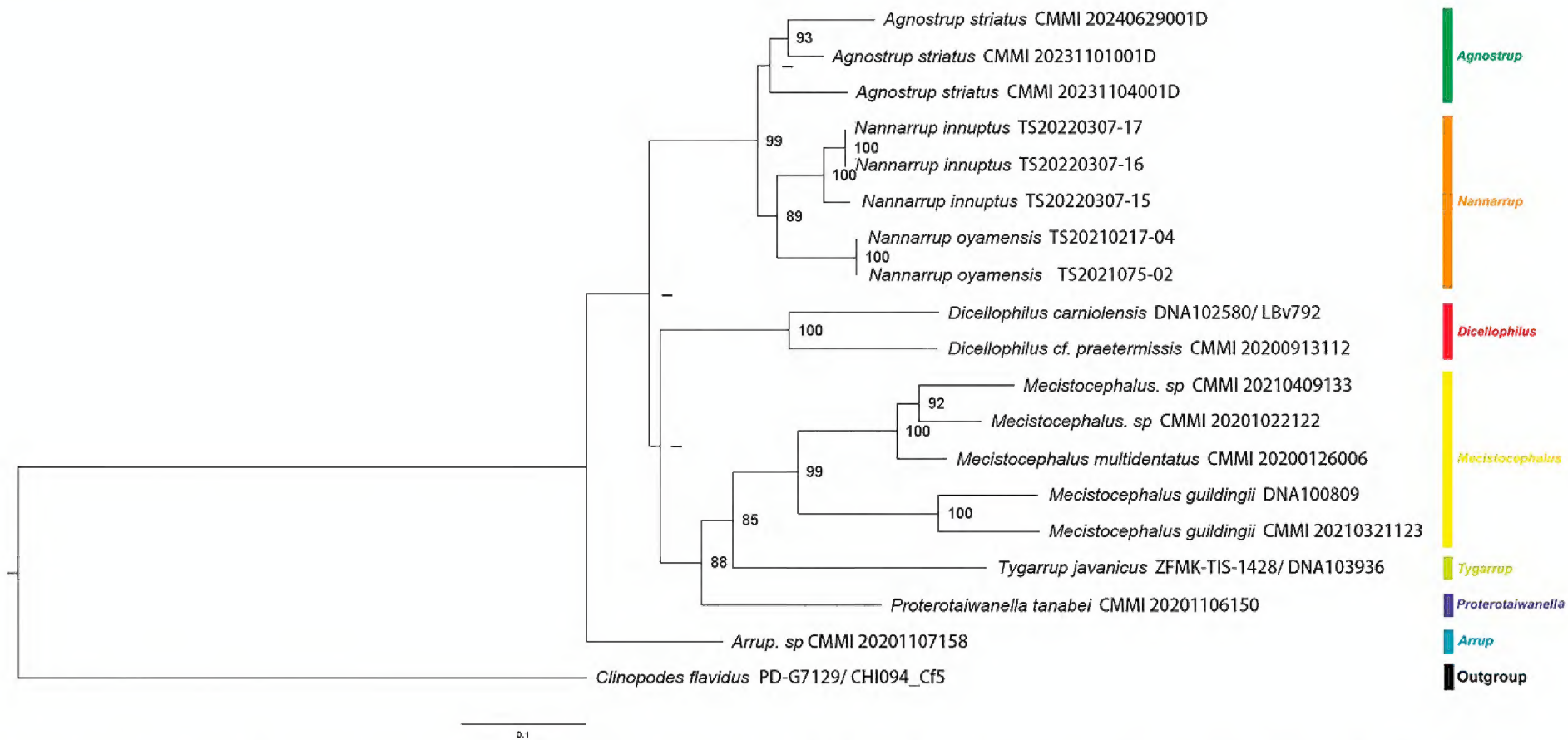


Figure 1. Maximum likelihood phylogenetic tree based on combined data for Mecistocephalidae. Values above the branches represent the bootstrap support (BS); BS < 70% are indicated as hyphens.

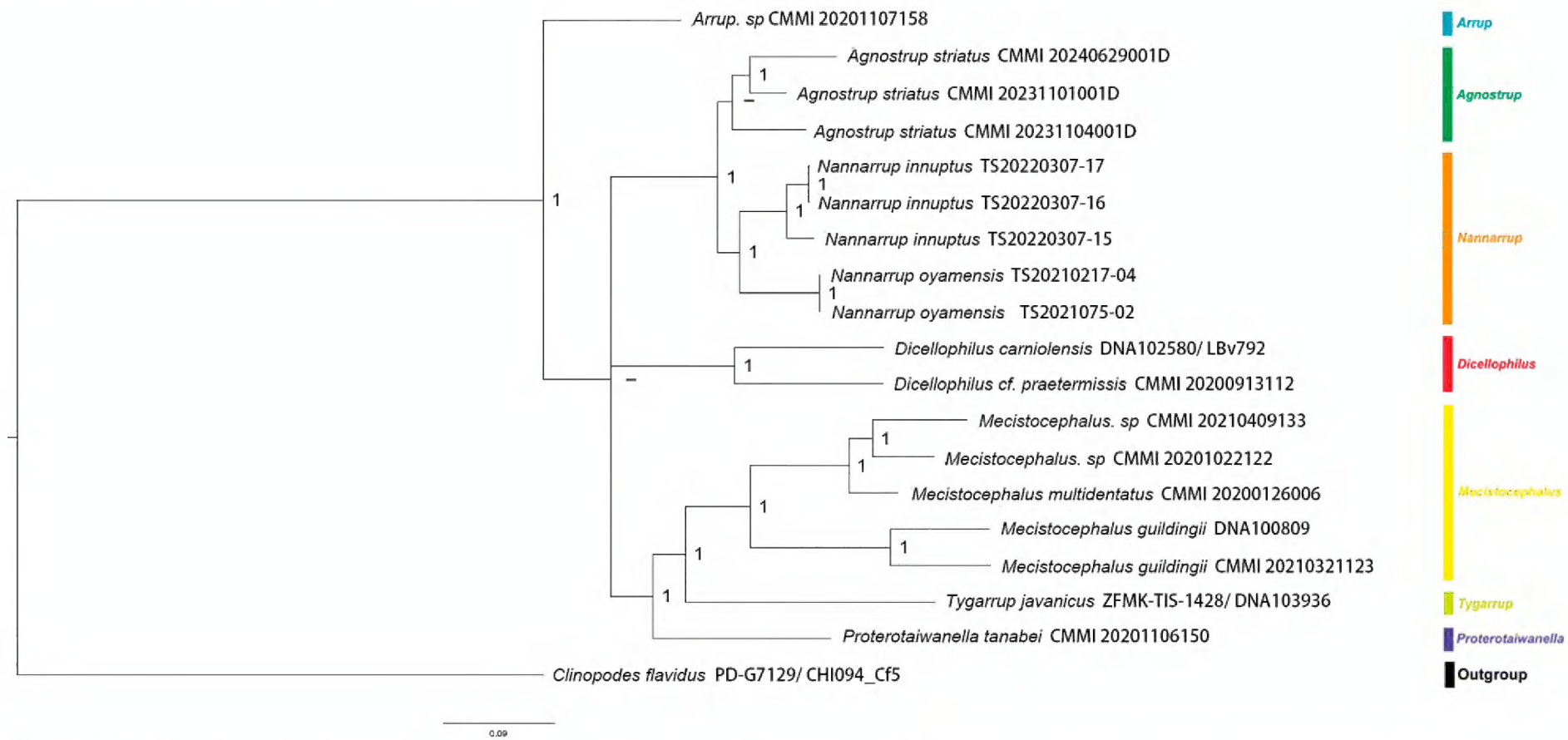


Figure 2. Bayesian inference based on combined data for Mecistocephalidae. Values above the branches represent the posterior probability (PP); PP < 0.9 is indicated as a hyphen.

characters (Foddai et al. 2003): Body inconspicuously tapering backwards; leg-bearing trunk uniform in color, without dark patches; cephalic plate only slightly longer than wide; usually 2 clypeal plagulae divided by a mid-longitudinal stripe, not covering more than posterior half of clypeus;

clypeal setae are mainly located in two laterally extended areas; buccae without setae; spiculum absent; internal margin of labral anterior ala reduced to a pointed end; posterior alae without longitudinal stripes; posterior margin of labral sidepiece sinuous, not fringed; coxosternum of first maxil-

Table 2. The list of specimens that were used in the phylogenetic analyses (the new sequences have been uploaded to GenBank).

Species	Voucher	Accession No.			Collection Location	References
		16s	28s	coi		
<i>Dicellophilus</i> cf. <i>praetermissus</i> Tsukamoto & Eguchi, 2024	CMMI 20200913112	PP788526	PP750339	PP737183	Huanren, Liao Ning, China	the present study
<i>Proterotaiwanella tanabei</i> Bonato, Foddai & Minelli, 2002	CMMI 20201106150	PP788520	PP750333	PP737177	Lian Yun Gang, Jiang Su, China	the present study
<i>Arrup</i> sp.	CMMI 20201107158	PP788525	PP750338	PP737182	Yan Cheng, Jiang Su, China	the present study
<i>Agnostrop striatus</i> (Takakuwa, 1949)	CMMI 20231104001D	PP788521	PP750334	PP737178	Chang Zhi, Shan Xi, China	the present study
	CMMI 20231101001D	PP788522	PP750335	PP737179	Ning Wu, Shan Xi, China	the present study
	CMMI 20240629001D	PQ213467	–	PQ222662	Ning Wu, Shan Xi, China	the present study
<i>Mecistocephalus</i> sp.	CMMI 20210409133	PP788523	PP750336	PP737180	Jing Shan, Hu Bei, China	the present study
	CMMI 20201022122	PP788527	PP750340	OR864655	Jian Shui, Yun Nan, China	Pan et. al. (2024)
<i>Mecistocephalus multidentatus</i> Takakuwa, 1936	CMMI 20200121006	PP788524	PP750337	PP737181	Heng Yang, Hu Nan, China	the present study
<i>Tygarrup javanicus</i> Attems, 1929	ZFMK-TIS-1428 DNA103936	HM453226	HM453286	KM491598	–	Murienne et al. (2010)
<i>Agnostrop oyamensis</i> (Tsukamoto,2022)	TS20210217-04	LC715630	LC715705	LC715555	Hinata, Isehara-shi, Kanagawa prefecture, Japan	Tsukamoto et al. (2022)
	TS20210725-02	LC715631	LC715706	LC715556	Hinata, Isehara-shi, Kanagawa prefecture, Japan	Tsukamoto et al. (2022)
<i>Agnostrop innuptus</i> (Tsukamoto,2022)	TS20220307-15	LC715484	LC715559	LC715634	Makigawa, Tsushimacho, Uwajima-shi, Ehime prefecture, Japan	Tsukamoto et al. (2022)
	TS20220307-16	LC715483	LC715558	LC715633	Makigawa, Tsushimacho, Uwajima-shi, Ehime prefecture, Japan	Tsukamoto et al. (2022)
	TS20220307-17	LC715482	LC715557	LC715632	Makigawa, Tsushimacho, Uwajima-shi, Ehime prefecture, Japan	Tsukamoto et al. (2022)
<i>Dicellophilus carniolensis</i> (C.L. Koch, 1847)	DNA102580 LBv792	HM453225	HM453285	KF569305	– –	Murienne et al. (2010) Bonato et al. (2014)
<i>Mecistocephalus guildingii</i> Newport, 1843	DNA100809	AY288728	HM453283	AY288747	–	Edgecombe and Giribet (2004); Murienne et al. (2010)
	CMMI 20210321123	PP788528	PP750341	PP737184	Yuan Yang, Yun Nan, China	the present study
<i>Clinopodes flavidus</i> Koch, 1847	PD-G7129 CHI094_Cf5	MZ427910	EU376008.1	MH816990	–	Peretti et al. (2022)

Table 3. Mean K2P genetic distance between the genus *Agnostrop* and other genera of Mecistocephalidae.

	<i>Tygarrup</i>	<i>Proterotaiwanella</i>	<i>Nannarrup</i>	<i>Mecistocephalus</i>	<i>Dicellophilus</i>	<i>Arrup</i>
C011	22.2%	20.5%	16.4%	18.9%	20.3%	21.9%
16S	26.9%	26.6%	18.1%	29.6%	26.8%	27.6%
28S	11.8%	12.0%	10.6%	11.7%	13.4%	10.6%

Table 4. Mean K2P genetic distance between the specimens of *Agnostrop striatus*.

Examined genus	Mean distance (Standard error)		
	C01	16S	28S
1. <i>Agnostrop</i>	13.3% (1.4%)	12.7% (1.5%)	2% (0%)

lae either divided and nonareolate or undivided and areolate, anterolateral corners virtually absent; coxosternum of second maxillae undivided or coxae connected by a membranous isthmus; groove from metameric pore and foram-

inal process reaching postero-external corner of coxosternum; reduced development of the telopodites of the second maxillae; forcipular tergum evidently wider than long, without a mid-longitudinal sulcus; cerrus absent; forcipular

Table 5. Morphological comparison of six species within the genus *Agnostrup* are based on the description and reference pictures published by Foddai et al. 2003; Tsukamoto et al. 2022; Takakuwa and Takashima 1949; Titova 1975; Uliana et al. 2007.

Characters	<i>A. paucipes</i> (Miyosi, 1955)	<i>A. striatus</i> (Takakuwa,1949)	<i>A.</i> <i>striganovae</i> (Titova, 1975)	<i>A. hoffmani</i> Foddai, Bonato, Pereira & Minelli, 2003	<i>A. innuptus</i> Tsukamoto, 2022	<i>A. oyamensis</i> Tsukamoto, 2022
body length	at least 20 mm	20–35 mm	over 30 mm	10.3 mm	7.0–12.0 mm	8.6 mm
Clypeus: setae on the areolate part (each side)	3	5–8	4–7	7	9	6–7
Clypeus: setae on the plagulae (each side)	8	3–6	5	0	0	0
Clypeus: insulae	absent	present	absent	absent	present	absent
lamellae on mandible	5	5	6	4	at least 4	at least 4
second maxillae: pretarsus	absent	rudimentary truncate claw	absent	short spine	short spine	short spine
Forcipular segment: article I	well-developed distal tooth	large tooth	large and blunt tooth	strong pigmented basal tooth	strong pigmented basal tooth	strong pigmented basal tooth
Forcipular segment: article II	without tooth	without tooth	small sharp tooth	without tooth	without tooth	without tooth
Forcipular segment: articleIII	without tooth	tubercle	small sharp tooth	without tooth	tubercle	tubercle not visible
Forcipular segment: tarsungulum	long basal tooth	large and sharp tooth	large tooth	slightly pigmented basal tooth	well-pigmented basal denticle	well-pigmented basal denticle
Ultimate leg-bearing segment: pretarsus	–	small tubercle	–	small spine	small tubercle	spine

trochanteropraefemur stout, with a distal tooth only; sternal mid-longitudinal sulci not furcate; number of pairs of legs 41. Differences between these two genera include: a frontal line present in *Agnostrup* while absent in *Nannarrup*; two small clypeal plagulae covering approximately one-sixth of the clypeus in *Nannarrup*, whereas the clypeal plagulae of *Agnostrup* cover one-half to most of their clypeus; the mandible in *Nannarrup* is provided with four well-developed pectinate lamellae, whereas 5–6 are present in *Agnostrup*. In contrast to the distinctions observed among other genera within the family Mecistocephalidae, these characters are not significant enough to serve as morphological characters for delineating them as two distinct genera. (The more detailed characteristics of the species within these two genera are presented in Table 5.)

Taxonomic account

Family Mecistocephalidae Bollmann, 1893

Agnostrup Foddai, Bonato, Pereira & Minelli, 2003.
Agnostrup Foddai, Bonato, Pereira & Minelli, 2003: 1254.
Agnostrup: Uliana, Bonato and Minelli 2007: 24.
Nannarrup Foddai, Bonato, Pereira & Minelli, 2003, syn. nov.

Type species. *Krateraspis striganovae* Titova, 1975 – by original designation.

Species included. *Agnostrup hoffmani* (Foddai, Bonato, Pereira & Minelli, 2003), comb. nov., *Agnostrup innuptus* (Tsukamoto, 2022), comb. nov., *Agnostrup oyamensis* (Tsukamoto, 2022), comb. nov., *Agnostrup*

paucipes (Miyosi, 1955), *Agnostrup striatus* (Takakuwa, 1949), and *Agnostrup striganovae* (Titova, 1975).

Diagnosis. Mecistocephalids with 41 leg-bearing segments; two clypeal plagulae separated by a mid-longitudinal stripe. Clypeal setae are arranged in a transversal band on the anterior part of the plagulae and on a medial part of the areolate clypeus; spiculum absent. Side-pieces of labrum are divided into anterior and posterior alae; the internal margin of each anterior ala is reduced to a point. Posterior alae with or without longitudinal stripes; posterior margin of labrum is not hairy. Mandible is provided with 4–6 pectinate lamellae. Coxosternum of the first maxillae is divided in the middle; coxosternum of the second maxillae is undivided. Telopodites terminals of the second maxillae with or without a claw. Forcipular trochanteropraefemur with one distal tooth pointing forward; forcipular articles II and III with or without teeth. Basal tooth of tarsungulum well developed. Forcipular tergum without median sulcus; sternal sulcus of trunk segments not furcate. 7–15 pores on the ventral surface of each coxopleuron; anal pores are present.

Remarks. *Agnostrup* differs from other genera of the Mecistocephalidae previously recorded in China due to its unusual forcipular teeth: the trochanteropraefemur with one well-developed distal tooth pointing forward and a well-developed basal tooth of the tarsungulum. *Agnostrup* differs from *Mecistocephalus* by the presence vs. absence of a pair of spicules projecting from the cephalic pleurites. It differs from *Proterotaiwanella* in the pattern of clypeal setae and sensilla and the finger-like processes of the labrum (Bonato et al. 2002). It differs from *Dicellophilus* by the latter having a labrum with longitudinal folds and

fringed by marginal bristles (Bonato et al. 2010). From *Tygarrup*, it differs in having an entire plagula lacking a mid-longitudinal areolate strip (Chao et al. 2020). Finally, it differs from *Arrup* in the unusual teeth of the forcipules and the later having entire coxosternite of the first maxillae, without a mid-longitudinal suture (Foddai et al. 2003).

Agnostrop clearly resembles *Nannarrup* morphologically and in geographical distribution. *Nannarrup* was established for a single species discovered in New York, USA, and is considered to have been introduced from western America or East Asia (Foddai et al. 2003). Nearly twenty years later, Tsukamoto et al. (2022) discovered two new *Nannarrup* species, *N. innuptus* Tsukamoto, 2022, and *N. oyamensis* Tsukamoto, 2022, from Japan based on morphological characteristics and DNA analysis, confirming the East Asian origin of this genus. These two genera both share unique characters of forcipular article I with one well-developed distal tooth and a tarsungulum with one large basal tooth (Foddai et al. 2003; Tsukamoto et al. 2022). Comparisons of the original descriptions of *Agnostrop* and *Nannarrup* reveal several differences between these two genera, such as the presence vs. absence of a frontal line, side-pieces of the labrum fully divided into two alae vs. only incompletely subdivided into an anterior and a posterior ala, the number of setae on the clypeal plagulae, the shape of the pretarsus of the second maxillae, the tooth vs. denticle of the forcipular tarsungulum, the absence vs. presence of claws on the second maxillae, along with different numbers of mandible pectinate lamellae. However, the side-pieces of the labrum are fully divided into two alae in *N. innuptus* and *N. oyamensis* (Tsukamoto et al. 2022), and claws of the second maxillae were confirmed to be present in *A. striatus* after examining the new specimens collected from Shanxi province in China. The size of the teeth on the forcipular tarsungulum is also variable among species within the genus *Nannarrup*; the type species *N. hoffmani* is described as “tarsungulum with a well-developed and slightly pigmented basal tooth,” which is quite similar to the *A. striatus* specimen. However, in *N. innuptus* and *N. oyamensis*, this characteristic is described as “tarsungulum with a well-pigmented basal denticle.” Additionally, we compared the size of the trochanteroprefemur tooth among the three species within *Nannarrup* and found that *N. innuptus* and *N. oyamensis* have a distal tooth on the trochanteroprefemur that is very similar in size to that of *A. striatus* but significantly larger than that of *N. hoffmani*.

Therefore, the only definite difference between these two genera is the cephalic plate of *Agnostrop*, which has a frontal line, while *Nannarrup* lacks one. However, all three *Nannarrup* species have a smaller body length (ca. 10 mm) compared with the four *Agnostrop* species (ca. 15–30 mm). The frontal line may be absent in small mecistocephalid species. Uliana et al. (2007) also described a small *Arrup* species, *A. lilliputianus* Uliana, Bonato & Minelli, 2007, with a body length of approximately 10 mm that lacks a frontal line. Considering the low genetic distances between *Nannarrup* and *Agnostrop* and

the morphologically insignificant characters for delineating them as two distinct genera, we consider *Nannarrup* and *Agnostrop* to be the same genus. Accompanied by these changes, three species previously belonging to the *Nannarrup* genus are transferred to *Agnostrop* as *A. hoffmani* (Foddai, Bonato, Pereira & Minelli, 2003), comb. nov., *A. innuptus* (Tsukamoto, 2022), comb. nov., and *A. oyamensis* (Tsukamoto, 2022), comb. nov.

Agnostrop striatus (Takakuwa, 1949)

Figs 3, 4

Taiwanella striata Takakuwa 1949: 51–69, fig 1.

Taiwanella striata: Wang 1996: 81–99.

Agnostrop striatus: Foddai et al. 2003: 1255.

Material examined. *Neotype* (this paper formally assigns): 1 male (CMMI 20231104002D), CHINA, Shanxi Province, Changzhi, Taihang Grand Canyon; 35.9450°N, 113.4520°E; 1280 m a.s.l.; 4 Nov. 2023; coll. Tianyun Chen, Jiabo Fan & Yuan Xiong.

Non-type specimens: 1 female (CMMI 20231104001D), same data as neotype • 1 female (20240629001D), CHINA, Shanxi Province, Ningwu County, Qingren Valley Scenic Area; 38.7980°N, 112.0365°E; 1798 m a.s.l.; 29 Jun. 2024; coll. Jiabo Fan & Yizhan Shi • 1 female (CMMI 20231101001D), CHINA, Shanxi Province, Xinzhou, Mt. Luyashan; 38.9166°N, 112.1031°E; 1910 m a.s.l.; 1 Nov. 2023; coll. Tianyun Chen, Jiabo Fan & Yuan Xiong.

Diagnosis. An *Agnostrop* species with areolate part of the clypeus bearing a smooth insulae on each side. Telopodites of second maxillae with or without a rudimentary truncate claw. Forcipular article I with one large distal tooth, articles II without tooth, articles III with one acute tooth, tarsungulum with one large basal tooth.

Re-description. [The original holotype data from Takakuwa and Takashima (1949) is provided in parentheses]: Body length 20–33 mm (35 mm). Head and forcipule segment dark red in color, rest yellow.

Cephalic plate (Fig. 3A, B): Head 1.5–1.7 times as long as wide, lateral margins slightly convergent backward; frontal line slight protuberance, almost parallel; some setae and punctate depressions in the anterior of back side.

Antennae (Fig. 3C): with 14 articles, the distal end slightly attenuate. Setae are scattered on articles I to VII, while on the remaining articles, the setae are denser and more uniform in size.

Clypeus (Fig. 3D): Clypeal ratio about 1.2; areolate part with 5–8 (5–7) setae, an evident smooth insulae on each side, insulae bearing 2 (1) setae. clypeal plagulae with 3–4 (4–5) setae and an irregular anterior margin.

Labrum (Fig. 3D): anterior ala triangular, medial margin reduced to a vertex; posterior margin of each side-piece straight, not crenulate, and without hair-like projections.

Mandible (Fig. 3E): bearing 5 well-developed lamellae; average intermediate lamella with approximately 5 (7) teeth, all teeth of similar size.

First maxillae (Fig. 3F): coxosternite divided by mid-longitudinal, anterior corners of coxosternum not projecting; each medial projection about 1.2 times as long as wide, with 3 setae, the distal lobe clavate; telopodite about 2–2.1 times as long as wide, without seta, the distal lobe attenuated.

Second maxillae (Fig. 3F): coxosternite medially undivided, without suture; article I of the telopodite about 2.3–2.4

times as long as wide; article III about 2–2.7 times as long as wide, with several setae; telopodites tri-articulate overreaching medial projections; and telopodites of first maxillae. The pretarsus in the form of a rudimentary truncate claw.

Forcipular segment (Fig. 4A–C): The exposed part of the coxosternite is as long as it is wide. anterior margin with shallow medial concavity and with one pair of denticles. Forcipules: the length-to-width ratio of article I is

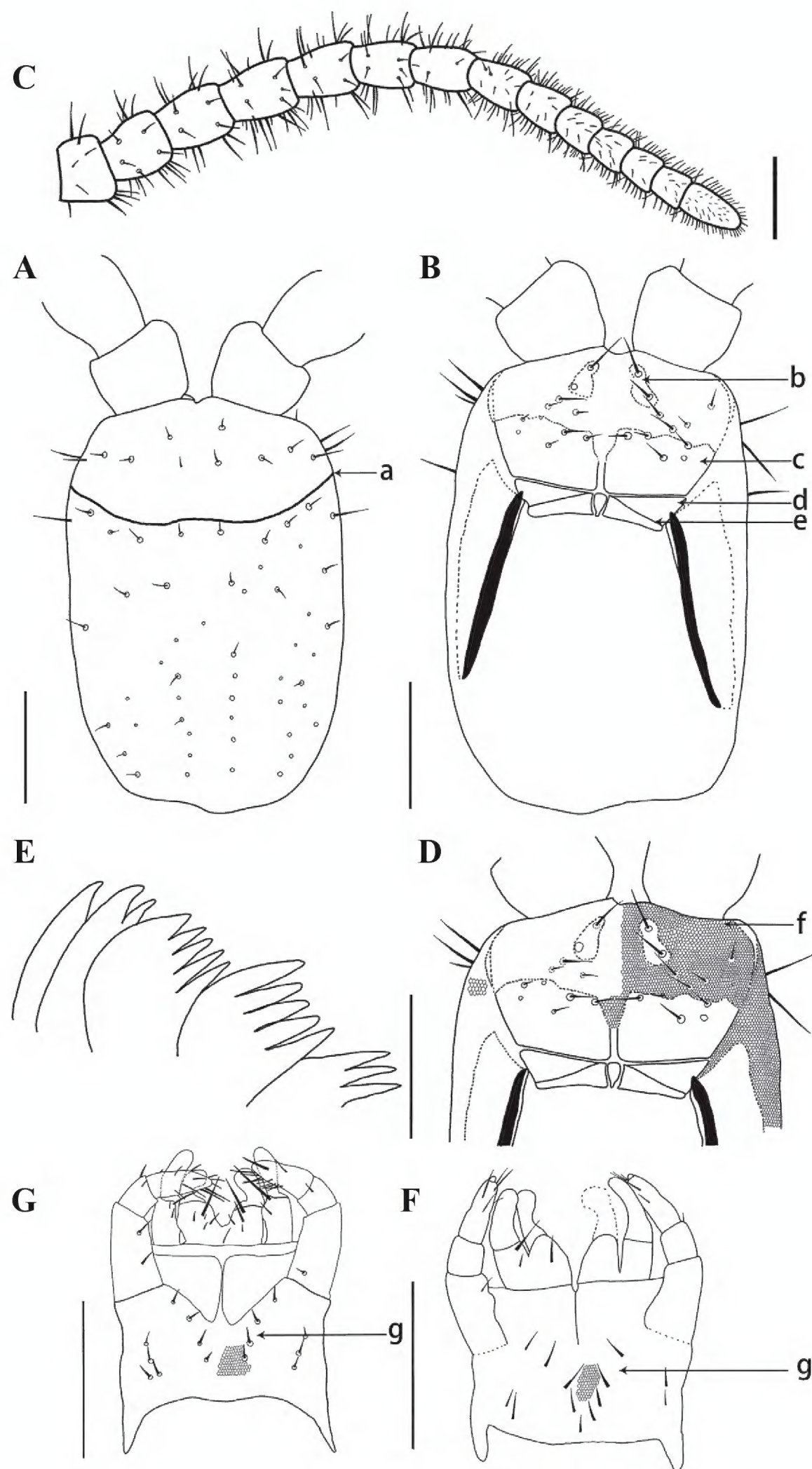


Figure 3. *Agnostrup striatus* (Takakuwa, 1949), (spm. CMMI 20231104002D). **A.** Cephalic plate, dorsal view; **B.** Clypeus, ventral view; **C.** Antenna, dorsal view; **D.** Cephalic plate, ventral view; **E.** Mandible; **F.** Maxillary complex, ventral view; **G.** Maxillary complex, ventral view (spm. CMMI 20231101001D). Arrowheads: a, frontal line; b, insulae; c, plagulae; d, posterior ala; e, transverse thickened line; f, clypeal areolate part; g, second maxillae coxosternite. Scale bar: 250 μ m.

greater than one with a well-developed tooth; articles II without tooth; articles III with one acute tooth; tarsungulum with a long basal tooth. Poison calyx only reaching the distal part of forcipular article I.

Leg-bearing segments (Fig. 4D, E): 41 leg-bearing segments; represented by very shallow mid-longitudinal thickening, anterior not furcate; no ventral glandular pores on each metasternite; the first pair of legs much smaller than the rest and the distributed bristles.

Ultimate leg-bearing segment (Fig. 4F–I): sternum sub-triangular, as long as wide; about 15 pores on each coxopleuron. Ultimate legs with or without a small apical tubercle covered with small spines.

Postpedal segments (Fig. 4F–I): The female and male gonopods are both distinct and biarticulate, with a few setae.

Remarks. The type material of the species *Taiwanella striata* (Takakuwa, 1949) is not available and is probably lost (Foddai et al. 2003). Similarly, Takashima (1954,

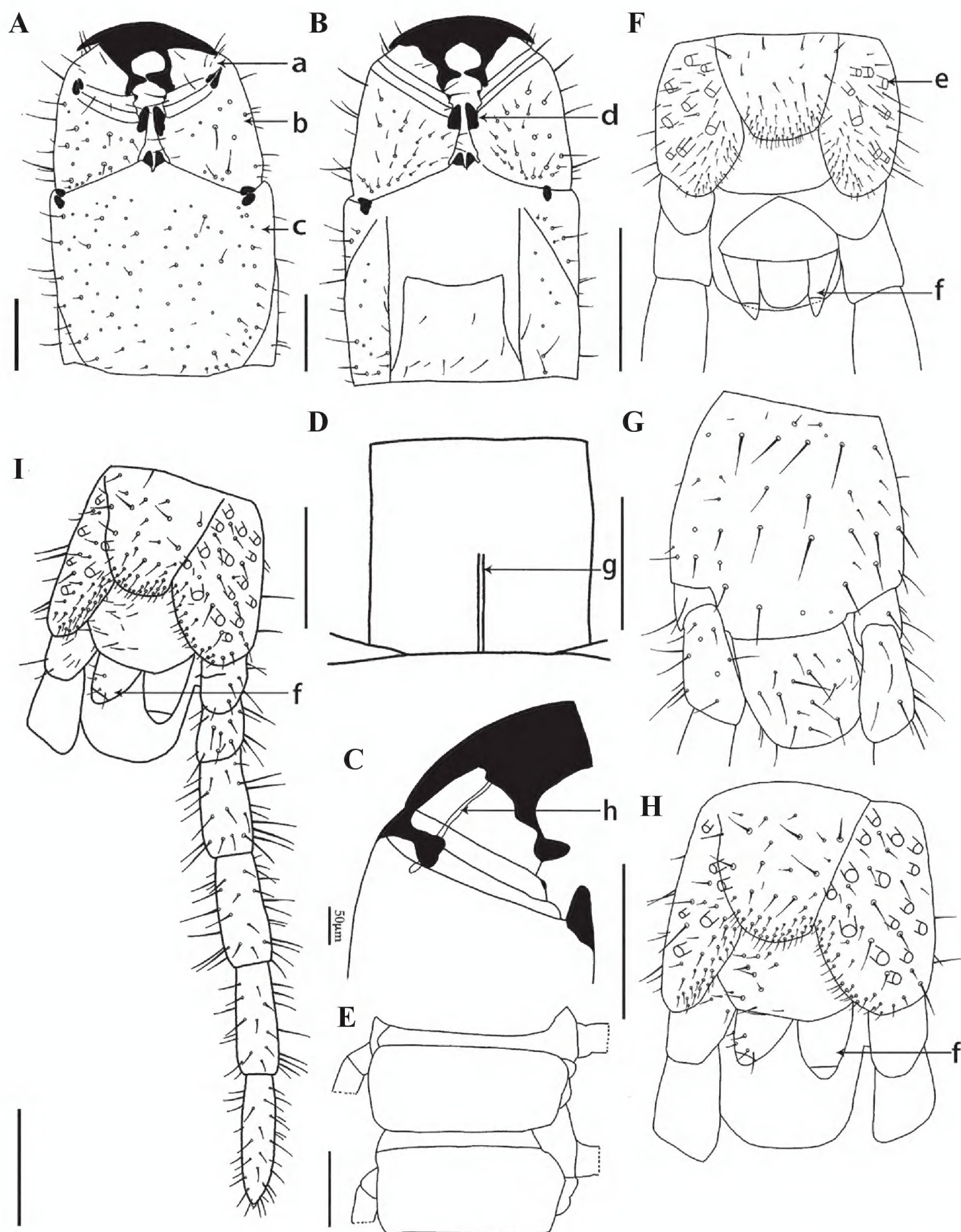


Figure 4. *Agnostrop striatus* (Takakuwa, 1949), (spm. CMMI 20231104002D). **A.** Forcipular segment, ventral view; **B.** Forcipular segment, dorsal view; **C.** A part of left forcipules, ventral view; **D.** Sternum of leg-bearing segment, ventral view; **E.** Tergite of leg-bearing segment, dorsal view; **F.** Ultimate leg-bearing segment, ventral view (spm. CMMI 20231104001D); **G.** Tergite of leg-bearing segment, dorsal view; **H.** Ultimate leg-bearing segment, ventral view; **I.** Ultimate leg-bearing segment and left leg, ventral view; **J.** Ultimate left leg. Arrowheads: a, forcipular tarsungulum; b, forcipular article I; c, forcipular coxosternite; d, tooth of forcipular article I; e, coxal pore; f, gonopod. Scale bar: 250 μ m.

1955) conducted a survey of the centipede-type specimens existing in Japan and did not find the holotype of *T. striatus*, speculating that it may have been destroyed during World War II. The type locality of *T. striata* is an unknown locality (Japanese phonetic; it is pronounced near Cinkaiji) of Shanxi, China. We were unable to match it to any present geographical name but collected four fresh specimens morphologically matching this species from Shanxi. Therefore, we designate the specimen that most closely matches the original description as the neotype for this species.

Among these, three specimens had a noticeable rudimentary truncate claw on the telopodites of the second maxillae, while one specimen clearly lacked the claw. The claw of the second maxillae was clearly described by Takakuwa and Takashima (1949), but Foddai et al. (2003) and Uliana et al. (2007) believed that the genus *Agnostrop* lacks claws on the telopodites of the second maxillae. We conducted meticulous morphological examinations, genetic distance, and molecular phylogenetic analyses on these samples, confirming that they all belong to the same species. The differences in this characteristic are identified as minor morphological variations (Fig. 5).

The original description by Takakuwa and Takashima (1949) clearly indicates several smooth insulae on the clypeus, with the third segment of the forcipular article III lacking teeth. This is notably different from the specimens we found in Shanxi: the forcipular article III with one acute tooth in dorsal view, each side of the clypeus bears a smooth insulae, with two setae on each insulae.

Distribution. China (Shanxi).

Discussion

Species recognition based on morphological analysis and DNA barcoding

The genus *Arrup* is nested at the base of Mecistocephalidae in both ML and BI analyses (PP = 1), which is inconsistent with the findings of Bonato et al. (2003, Fig. 4). Bonato et al. (2003, Fig. 4) present an ML tree based on the number of leg-bearing segments, indicating that *Arrup*, *Agnostrop*, and *Nannarrup* belong to the same clade, the subfamily Arrupinae. Meanwhile, the results for *Proterotaiwanella*, which in our trees forms a clade with *Tygarrup* and *Mecistocephalus* (PP = 1; BS = 85%), were also inconsistent with previous morphological findings.

There are too many shared morphological characteristics between *Agnostrop* and *Nannarrup*, and the few differences, including the number of setae on the clypeal plagulae and the size of the forcipular tarsungulum, are too subtle. Compared to other genera in the family Mecistocephalidae, these characters are not significant enough to delineate them as two distinct genera. Additionally, the genetic distance between *Agnostrop* and *Nannarrup* is

smaller than the distances between *Agnostrop* and other genera, indicating that they could also be considered the same genera genetically.

In addition to the genera *Nannarrup*, *Agnostrop*, and *Arrup*, *Partygarrupius moiwaensis* (Takakuwa, 1934) also belongs to the Arrupinae Chamberlin, 1912, and is an endemic species of Hokkaido Island, Japan. It has 41 pairs of legs, buccae without setae, and no spiculum. The coxosternum of the first maxillae is divided, while that of the second maxillae is undivided. The forcipular trochanteropraemur has only a distal tooth, and the tarsungulum has only one pointed tooth. The forcipular tergum lacks a median sulcus, and the sternal rhachides are not anteriorly furcate. Based on these similar morphological characteristics, we hypothesize that *Partygarrupius moiwaensis* (Takakuwa, 1934) may form a sister group with *Nannarrup* and *Agnostrop* in the phylogenetic tree. However, unlike *Nannarrup* and *Agnostrop*, it possesses an entire clypeal plagula, without a mid-longitudinal areolate stripe and extending along the lateral margins of the clypeus. This feature is similar to that of *Tygarrup*.

Based on morphological analysis and DNA barcoding, the genus *Agnostrop* now comprises six species. Detailed comparisons of species' morphological characteristics can be found in Table 4.

Distribution of the genus *Agnostrop*

Most species of Arrupinae are confined to specific geographic locations, but the genus *Agnostrop*, with its broad distribution from Shanxi Province and northeastern China to Japan and New York, USA, exhibits a clear dispersal pattern. Specimens of *Agnostrop* spp., which can be as small as 1 cm, are easily transported during the movement of plants (Foddai et al. 2003). The presence of *A. hoffmani* in New York City is confirmed to be a result of human introduction. Another example of human-mediated introduction is *Tygarrup intermedius* Chamberlin, 1914, the type species of *Tygarrup*, originally described from British Guyana but actually taken in Washington in pots of plants imported from British Guyana (Chamberlin, 1914). Similarly, *Tygarrup javanicus* Attems, 1929, a centipede native to Southeast Asia and widely distributed in the Indochinese Peninsula, Java, and other regions, has been found in the Seychelles and Mauritius islands. In recent years, *Tygarrup javanicus* has been recorded in the Hawaiian Islands, suggesting its spread through human activity. This introduction is not unprecedented, as similar cases have been reported in Kew Gardens, London, and Vienna, Austria (Bonato et al. 2004).

The *Agnostrop* genus, distributed in Japan, Shanxi Province in China, and the Sikhote-Alin mountains in Russia, may indicate its adaptation to temperate and subarctic climates. Although these regions are geographically dispersed, they share similar climate zones and ecological environments, particularly forested mountainous

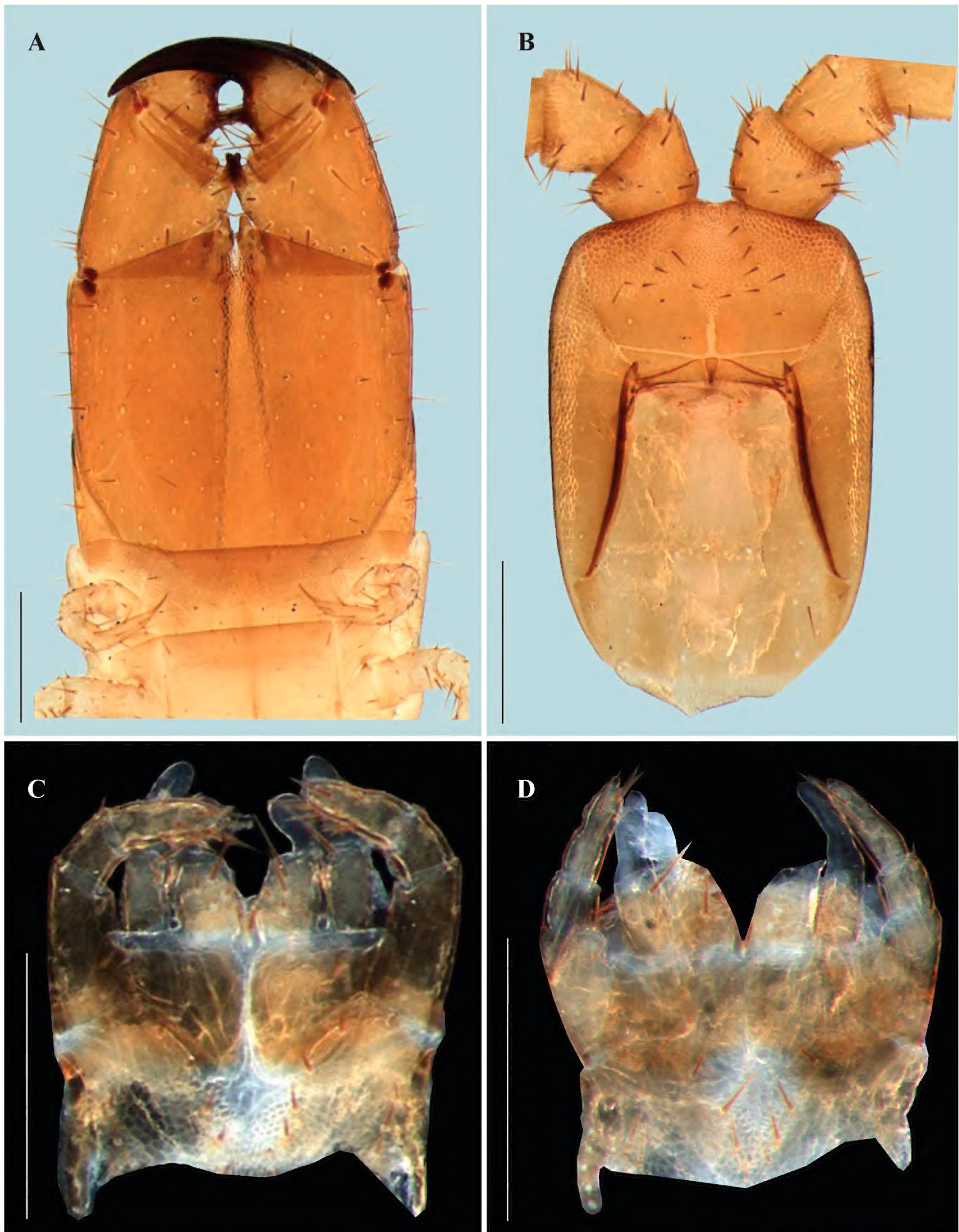


Figure 5. *Agnostrop striatus* (Takakuwa, 1949). **A.** Forcipular segment, ventral view (spm. CMMI 20231104001D); **B.** Clypeus, ventral view (spm. CMMI 20231104001D); **C.** Maxillary complex, ventral view (spm. CMMI 20231101001D); **D.** Maxillary complex, ventral view (spm. CMMI 20231104002D). Scale bar: 250 μ m.

terrain. This similarity likely provides stable habitat for the genus, enabling it to survive and thrive in these areas. The distribution of the *Agnostrop* genus extends from Shanxi Province and northeastern China to Japan, further demonstrating its notable geographic dispersal pattern. This wide distribution may be driven by several factors. The climate and ecological environments of Shanxi Prov-

ince and northeastern China are somewhat similar to those of Japan, particularly within temperate forest ecosystems, which may offer suitable habitats for the natural dispersal of *Agnostrop*. Additionally, the geographic proximity between Japan and northeastern China may facilitate the dispersal of the genus through natural pathways such as wind, river basins, or cross-ocean biological dispersal.

Key to the species of *Agnostrop*

- 1 With evident smooth insulae on the clypeus..... 2
- Without evident smooth insulae on the clypeus..... 3
- 2 Two large clypeal plagulae covering approximately one-half of the clypeus..... *A. striatus* (Takakuwa, 1949)
- Two small clypeal plagulae covering approximately one-sixth of the clypeus..... *A. innuptus* (Tsukamoto, 2022)
- 3 Clypeal plagulae with additional smooth or weak areolation area along posterior part of paraclypeal sutures
..... *A. hoffmani* (Foddai, Bonato, Pereira & Minelli, 2003)
- Clypeal plagulae without additional smooth or weak areolation area along posterior part of paraclypeal sutures
..... 4
- 4 Telopodites of second maxillae with a short spine..... *A. oyamensis* (Tsukamoto, 2022)
- Telopodites of second maxillae without a short spine..... 5
- 5 Forcipular articles II and III with a tubercle *A. striganovae* (Titova, 1975)
- Forcipular articles II and III without a tubercle *A. paucipes* (Miyosi, 1955)

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